Associations of the fatty liver and hepatic steatosis indices with risk of cardiovascular disease: Interrelationship with age

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Abbreviation: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; CT, computed tomography; CVD, cardiovascular disease; GGT, gamma-glutamyltransferase; FLI, fatty liver index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-estimated insulin resistance; HR, hazard ratio; hsCRP, high sensitivity C-reactive protein; HSI, hepatic steatosis index; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; PREVEND, Prevention of Renal and Vascular End-stage Disease; SBP, systolic blood

pressure; STROBE, STrengthening the Reporting of OBservational studies in Epidemiology; TG, triglyceride; UAE, urinary albumin excretion; WC, waist circumference;

ABSTRACT

Background: The fatty liver index (FLI) and the hepatic steatosis index (HSI), are biomarker-based algorithms developed as proxies for non-alcoholic fatty liver disease (NAFLD). We assessed associations of FLI and HSI with cardiovascular disease (CVD) risk.

Materials and methods: The FLI and HSI were estimated at baseline in the PREVEND cohort involving 6340 participants aged 28-75 years without pre-existing CVD.

Results: During a median follow-up of 10.5 years, 631 CVD events occurred. In age- and sex-adjusted analysis, the hazard ratio (HR) (95% CI) for CVD comparing FLI ≥ 60 versus FLI < 30 was 1.53 (1.25-1.88); which was attenuated to 0.89 (0.70-1.13) on adjustment for conventional cardiovascular risk factors. The association remained absent after additional adjustment for potential confounders 0.85 (0.65-1.11). Comparing HSI > 36 versus HSI < 30, the corresponding adjusted HRs were 1.29 (1.02-1.65), 0.84 (0.65-1.09) and 0.79 (0.55-1.13) respectively. Subgroup analyses suggested a positive association in younger participants (< 50 years) for FLI and inverse associations in older participants (≥ 50 years) for both indices (*P* for interaction for all = 0.001).

Conclusion: Current data suggest age interactions in the association of NAFLD (as assessed by FLI or HSI) with CVD risk in a general Caucasian population.

Keywords: Fatty liver index; hepatic steatosis index; non-alcoholic fatty liver disease; cardiovascular disease; risk factor

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis with varying degrees of necroinflammation and fibrosis and which develops in the absence of substantial alcohol intake.[1] First described in 1980,[2] it represents a spectrum of liver disease ranging from simple hepatic steatosis to nonalcoholic steatohepatitis, fibrosis, and eventually cirrhosis.[1] Nonalcoholic fatty liver disease is now more common than alcoholic liver disease and is emerging as the most common cause of chronic liver disease in the developed world.[1, 3] In the absence of the reference standard - liver biopsy - which has well-known limitations such as invasiveness and sampling variability,[4] the diagnosis of NAFLD is commonly based on (i) imaging techniques [i.e., ultrasonography, computed tomography (CT) scan, or magnetic resonance imaging (MRI)] confirming the presence of fat infiltration of the liver and (ii) exclusion of other liver diseases of other aetiology.[5] However, these imaging techniques are associated with high costs and are not suitable for large-scale population-based epidemiological studies. Elevated alanine aminotransferase (ALT) has frequently been used as a biochemical surrogate for NAFLD diagnosis, however it is common to diagnose NAFLD in patients with normal ALT levels using ultrasonography or histology.[3, 6] A number of biomarker-based indices have been developed to aid the diagnosis of fatty liver disease. The fatty liver index (FLI) is based on body mass index (BMI), waist circumference (WC), triglycerides (TGs), and gamma-glutamyltransferase (GGT).[7] The hepatic steatosis index (HSI) is also a simple algorithm that is based on three variables [Alanine aminotransferase/aspartate aminotransferase ratio, BMI, and diabetes].[8] Both indices have been reported to have good diagnostic accuracies.[7-9]

Nonalcoholic fatty liver disease is associated with an increased risk of mortality, with CVD being the most common cause among NAFLD patients.[10, 11] There is a rapidly growing body of evidence that supports the existence of a strong link between NAFLD and risk of CVD. During the past few decades, a number of prospective studies have reported on the associations between NAFLD and cardiovascular risk [10-16] However, the variability in study designs, small sample sizes, short follow-up durations, insufficient adjustment for established cardiovascular risk factors, and inconsistent results, limit the

validity and generalisation of these findings. Whilst some studies used retrospective cohort designs with small patient populations;[10, 11, 15, 17] other studies were conducted in selected populations such as participants with pre-existing type 2 diabetes or vascular disease;[12, 13, 18] or studies used elevated liver enzyme levels as surrogate markers of NAFLD.[19] Some studies observed associations of NAFLD with risk of CVD,[14] and others showed no associations at all,[11, 15, 16] leaving great uncertainty regarding the nature of the association. Reviews of the literature have also reached conflicting conclusions.[20, 21] Evaluation of this association is important, because NAFLD is a global public health burden and an emerging risk factor for CVD.

Our aim was to assess the prospective association of NAFLD (as estimated by these two indices - FLI and HSI) with risk of CVD, using a large population-based sample of 6340 participants free from pre-existing CVD at baseline.

2. Materials and methods

2.1. Study design and participants

This report was conducted according to STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for reporting observational studies in epidemiology (**Supplementary material 1**).[22]

The study population comprised a representative sample of inhabitants aged 28-75 years living in the city of Groningen, the Netherlands, who were recruited into the Prevention of Renal and Vascular End-stage Disease (PREVEND) prospective cohort study; which was designed to investigate the natural course of urinary albumin excretion and its relation to renal and cardiovascular disease. Details of the study design and recruitment of participants have been described in detail elsewhere.[23, 24] In brief, of all inhabitants invited for pre-screening, 40,856 responded. After exclusion of patients with type 1 diabetes mellitus and pregnant females, 11,162 subjects were deemed eligible. Of these, 8,592 participated (77% participation rate) and constituted the actual PREVEND cohort. Baseline measurements were performed between 1997 and 1998. Participants with a prevalent history of CVD, renal disease, or malignancy were excluded in the

present analysis. We also excluded participants with excessive alcohol use (defined as four or more drinks per day) and those on medication for treating hyperlipidemia, which left a final cohort of 6340 subjects with non-missing information on relevant components for the two exposures, CVD risk markers, and outcomes for the present analysis. The derivation of the analytic sample is reported in **Supplementary material 2**. The medical ethics committee of the University Medical Center Groningen duly approved the PREVEND study, which complies with the Declaration of Helsinki. Each participant provided written informed consent for voluntary participation, which was documented in a consent form.

2.2. Risk factor assessment

Plasma glucose measurements were made using dry chemistry (Eastman Kodak, Rochester, New York). Blood lipid [total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides], high sensitivity C-reactive protein (hsCRP), serum creatinine, and serum cystatin C were measured using standard laboratory methods, which have been described in previous reports. High-density lipoprotein cholesterol was measured with a homogeneous method (direct HDL, AEROSETTM System, Abbott Laboratories, Abbott Park, USA).[25] Triglycerides were measured enzymatically. Total cholesterol and plasma glucose were assessed using Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, New York, USA). Urinary albumin excretion (UAE) was calculated as the mean of two 24-hour urine collections. Serum liver aminotransferase activities were measured using the standardized kinetic method with pyridoxal phosphate activation (Roche Modular P; Roche Diagnostics, Mannheim, Germany) according to the recommendations of the International Federation of Clinical Chemistry.[26, 27] Serum GGT activity was measured by an enzymatic colorimetric method (Roche Modular P; Roche Diagnostics, Mannheim, Germany). Total bilirubin was measured by a colorimetric assay (2,4-dicholoraniline reaction; Merck MEGA, Darmstadt, Germany). Diabetes was defined as a fasting glucose level of ≥ 7.0 mmol/L, a nonfasting glucose level of ≥ 11.1 mmol/L and/or use of glucose lowering medication according to self-report or to pharmacy data.[28]

2.3. Measures of NAFLD

The FLI was calculated based on the report by Bedogni and colleagues [7] using the following formula:

$$FLI = [e^{(0.953 \times \ln(TG) + 0.139 \times BMI + 0.718 \times \ln(GGT) + 0.053 \times WC - 15.745)}] / [1 + e^{(0.953 \times \ln(TG) + 0.139 \times BMI + 0.718 \times \ln(GGT) + 0.053 \times WC - 15.745)}] \times 100$$

The FLI ranges from 0 to 100, with FLI < 30 ruling out (sensitivity = 87%) and FLI ≥ 60 ruling in fatty liver disease (specificity = 86%) with a good diagnostic accuracy of 0.84 (95% CI: 0.81-0.87).

The HSI was estimated using the following formula as reported by Lee and colleagues:[8]

HSI = 8 x ALT/AST ratio + BMI (+2, if diabetes; +2, if female); with HSI values < 30 and > 36 ruling out and ruling in fatty liver respectively and has a diagnostic accuracy of 93.1%.

2.4. Endpoint ascertainment

We included all first-onset composite CVD events that occurred from study enrollment through to 01-01-2011. Information on the dates and causes of death were ascertained by record linkage with the Dutch Central Bureau of Statistics, whiles information on hospitalization for cardiovascular morbidity was received from PRISMANT; which is the Dutch national registry of hospital discharge diagnoses. The validity of information from this database has been shown to be reliable, with 84% of primary diagnoses and 87% of secondary diagnoses matching the diagnoses recorded in patients' charts.[29] Outcomes were coded according to the *International Classification of Diseases*, Ninth Revision (ICD-9) until 01-01-2009. After this date, ICD-10 codes were employed. First-onset CVD was defined as the combined endpoint of acute myocardial infarction, acute and subacute ischemic heart disease, coronary artery bypass grafting or percutaneous transluminal coronary angioplasty, subarachnoid hemorrhage, intracerebral hemorrhage, other intracranial hemorrhage, occlusion or stenosis of the precerebral or cerebral arteries, and other vascular interventions such as percutaneous transluminal angioplasty or bypass grafting of peripheral vessels and aorta.

2.5. Statistical analysis

The analyses were pre-specified to exclude participants with a history of CVD at baseline. Skewed variables such as ALT, AST, triglycerides, hsCRP, creatinine, UAE, and total bilirubin were natural log-transformed to approximate normal distributions. Descriptive analyses were performed to summarize baseline characteristics of participants according to categories of the FLI and HSI. Associations of FLI and HSI with CVD risk were evaluated using Cox regression hazards models, after confirmation of assumptions of proportionality of hazards. Hazard ratios were progressively adjusted for age and sex; other established CVD risk factors [age, sex, smoking status, history of diabetes, systolic blood pressure (SBP), total cholesterol, and HDL-C]; potential confounders such as alcohol consumption, glucose, and UAE; and further for triglycerides (for HSI), total bilirubin, hsCRP, and the homeostasis model assessment-estimated insulin resistance (HOMA-IR). We performed subgroup analyses using interaction tests to assess statistical evidence of any differences in HRs across categories of pre-specified individual characteristics [such as age, sex, smoking status, alcohol consumption, history of diabetes, BMI (obese versus non-obese), history of hypertension, total cholesterol (hypercholesterolemia versus non-hypercholesterolemia), HDL-C (low to average versus optimal levels), hsCRP (low to average versus high cardiovascular risk[30]), UAE, and total bilirubin]. All statistical analyses were conducted using Stata version 14 (Stata Corp, College Station, Texas) and 2-sided *P*-values < 0.05 were considered statistically significant.

3. Results

3.1. Baseline characteristics

Overall, the mean age at baseline of the 6340 participants eligible for the present study was 48 (SD 12) years and 54% were women. During a median (interquartile range) follow-up of 10.5 (9.6-10.8) years, 631 incident CVD events were recorded. Baseline descriptive characteristics of the participants according to categories of the FLI and HSI are shown in **Tables 1 and 2.** Individuals in the higher FLI categories were older, more likely to have diabetes, and generally had adverse levels of cardiometabolic parameters

(anthropometric indices, blood pressure, lipids, fasting glucose, hsCRP, and renal function markers) compared to the lowest category of FLI (< 30). A similar pattern was observed for categories of the HSI. **Figure 1** shows proportions of participants who developed incident CVD in the categories of FLI and HSI.

3.2. FLI and risk of incident CVD

In age- and sex-adjusted analysis, compared to the reference category of FLI (< 30), the HR for CVD for individuals in the top FLI category (≥ 60) was 1.53 (95% CI: 1.25 to 1.88; *P*<0.001). The corresponding HR was attenuated to 0.89 (95% CI: 0.70 to 1.13; *P*=0.354) after additional adjustment for smoking status, history of diabetes, SBP, total cholesterol, and HDL-C, and this remained consistent on further adjustment for alcohol consumption, fasting glucose, and UAE; as well as total bilirubin, hsCRP, and HOMA-IR (**Table 3**). The HRs generally did not vary significantly by levels or categories of several clinically relevant characteristics, except for a highly significant interaction by age (*P* for interaction = 0.001). Remarkably, a positive association was in younger individuals (< 50 years) compared with an inverse association in older individuals (≥ 50 years) (**Figure 2**). To evaluate if the age interaction on the association could be due to reverse causation, we carried out subsidiary analysis that excluded first two years of follow-up. The findings still showed significant evidence of an age interaction; a positive association was observed in younger individuals 1.67 (95% CI: 1.07 to 2.61; *P*=0.024) compared to a less robust inverse association in older individuals 0.81 (95% CI: 0.60 to 1.08; *P*=0.156) (*P* for interaction = 0.006).

3.3. HSI and risk of incident CVD

Compared to the reference category of HSI (< 30), the HR for CVD for individuals in the top HSI category (> 36) was 1.29 (95% CI: 1.02 to 1.65; *P*=0.035) in age- and sex-adjusted analysis. In additional adjustment for smoking status, history of diabetes, SBP, total cholesterol, and HDL-C, the corresponding HR was attenuated to 0.84 (95% CI: 0.65 to 1.09; *P*=0.180) and this remained persistent on further

adjustment for potential confounders (**Table 4**). In subgroup analyses, there were significant interactions by age (*P* for interaction = 0.001), smoking status (*P* for interaction = 0.008), and hsCRP (*P* for interaction = 0.05). Inverse associations were seen for older individuals (≥ 50 years), current and former smokers, and individuals with hsCRP > 3.0 mg/l (**Figure 1**). In subsidiary analyses that excluded first two years of follow-up, subgroup analysis still showed evidence of an inverse association among older individuals 0.69 (95% CI: 0.49 to 0.97; *P*=0.032) (*P* for interaction = 0.003).

4. Discussion

4.1. Key findings

In this large-scale population-based study of individuals without a history of CVD at baseline, we observed no association between NAFLD (as assessed by FLI and HSI) and the risk of CVD in analyses adjusted for established risk factors and further for potential confounders and mediators. The null associations remained generally consistent across several clinically relevant subgroups, except for significant interactions by age, blood pressure, smoking status, renal function, and hsCRP. Using FLI as an index of NAFLD, an inverse association was seen in older individuals compared to a positive association in younger individuals. A similar pattern of effect modification by age was also observed when HSI was used as a proxy for NAFLD. Different and even opposing associations were also seen for smokers versus non-smokers.

4.2. Comparison with previous work

A number of previous studies have assessed the association of FLI with the risk of incident type 2 diabetes and have demonstrated strong positive associations.[9, 31] Yadav and colleagues have also recently shown that the FLI significantly improves diabetes risk prediction.[32] We are however unable to directly compare the current findings with previous work, as no prospective study to date has evaluated the association of the FLI or the HSI with the risk of CVD. The current results seem to be in contrast with those of prospective cohort studies that have reported on NAFLD (as diagnosed by imaging or histology)

and the risk of CVD. However, given the limitations of some of these previous study designs, the inconsistent evidence, and controversy surrounding the relationship between NAFLD and CVD, this may probably not be the case. Targher and colleagues using a prospective outpatient cohort showed NAFLD (diagnosed by ultrasonography) to be independently associated with fatal and non-fatal CVD events; however the cohort comprised selected patients with type 2 diabetes.[13] In a prospective cohort of apparently healthy Japanese men and women, NAFLD as diagnosed using ultrasonography, was demonstrated to be independently associated with risk of CVD; however, a main limitation of this study was that the incidence of CVD was assessed by self-administered questionnaires.[14] Pisto and colleagues using a Finnish cohort comprising mainly patients with established hypertension, showed NAFLD to be associated with fatal and non-fatal CVD in unadjusted analysis.[33] The results of Zeb and colleagues using the Multi-Ethnic Study of Atherosclerosis, suggested NAFLD (as diagnosed using non-enhanced CT scan) to be independently associated with risk of CVD; however, a composite endpoint of nonfatal coronary disease and all-cause mortality events was used.[34]

4.3. Possible explanations for findings

In line with established evidence which shows that NAFLD is associated with an increased risk of type 2 diabetes; accruing evidence suggests that NAFLD may also be involved in the pathogenesis of CVD. The putative underlying mechanisms linking increased cardiovascular risk with NAFLD is believed to originate from the expanded and inflamed visceral adipose tissue. The liver in its necroinflammatory form - non-alcoholic steatohepatitis – releases a vast array of pro-atherogenic and pro-inflammatory factors which are potentially involved in the development of insulin resistance and atherogenic dyslipidemia.[21, 35, 36] Insulin resistance is a pathogenic factor which plays a major role in the development of both the metabolic syndrome and CVD.[37] Nonalcoholic fatty liver disease is also associated with decreased plasma levels of adiponectin,[38] an adipose-secreted cytokine with anti-atherogenic properties and which has been shown to independently predict CVD.[39] However, despite the emerging evidence implicating a potential pathogenic role of NAFLD in the development of CVD; prospective observational cohorts

have been unable to consistently demonstrate a robust and independent association. Given the limited study designs of previous studies that have reported positive associations, there is a possibility that these associations demonstrated could be due to the effects of selection bias, reverse causation, and/or residual confounding. Nonetheless, the current results also bring into question the reliability of the FLI and HSI as suitable proxies for NAFLD. In the absence of liver biopsy, which is the gold standard for the diagnosis of fatty liver disease, these two scoring systems - which employ easy-to-measure variables – have been shown to detect fatty liver disease with considerable accuracy in comparison with ultrasonography.[7, 8] In addition, these indices have been externally validated in several populations and shown to have moderate diagnostic accuracy for steatosis in these populations.[40, 41] However, the FLI and HSI cannot substitute liver biopsy or MRI [41, 42] and only serve as surrogate measures of NAFLD. Overall, the evidence base for the association between NAFLD and CVD risk is still weak and further investigation is required. In our findings, we observed evidence of effect modification by a number of clinically relevant baseline characteristics; of which the most striking was age. Given the low event rate in some of the subgroups and the multiple statistical tests for interaction conducted, some of these observations should be interpreted with caution and require replication in further studies. For age, a consistent inverse association was seen in older individuals for both indices, whiles a positive association was observed in younger individuals for FLI. The age differences in the associations remained consistent in sensitivity analyses that excluded the first two years of follow-up and adjusted for other potential confounders. There is a possibility that the age interactions in the associations (inverse associations in participants who were ≥ 50 years) might due to the effect of exclusion at baseline of participants with prevalent history of CVD, renal disease, malignancy, and lipid medication; who were older and were more likely to have NAFLD. The findings might also reflect emerging evidence that ALT (commonly used as a surrogate measure of NAFLD) is an indicator of aging, sarcopenia, and frailty,[43, 44] which are associated with an increased risk of CVD.[45] Age has been suggested to play a role in mediating the association between ALT and CVD.[46] Indeed, we and others have shown that age modifies the relationship between ALT and CVD risk.[44, 46] Our findings may also reflect the paradoxical relationships between the aminotransferases,

diabetes, and CVD; which we and others have previously reported.[46, 47] These theories are biologically plausible but are speculative and therefore further research is needed to clarify these mechanistic pathways.

4.4. Study strengths and limitations

Several strengths of the current study merit consideration. To our knowledge, this is the first comprehensive epidemiological investigation of the prospective association between CVD risk and NAFLD using two validated indices. Our analyses was based on large-scale epidemiological data comprising of participants representative of the general population with over a decade of follow-up and usage of validated CVD events. To minimize possibilities of reverse-causation bias, this current study was designed to involve individuals free of clinically evident prior vascular disease or other chronic disorders at baseline. Potential bias due to reverse causation which could have been responsible for the age differences in the associations was minimised by excluding the first two years of follow-up in sensitivity analysis. We had measurements of several lifestyle and biological markers which enabled comprehensive adjustment for potential confounding. Limitations of the current analyses include (i) the potential residual confounding due to unmeasured confounders and errors in measurements of potential confounders; (ii) inability to differentiate between alcoholic fatty liver disease and NAFLD with certainty; though we excluded subjects with excessive alcohol consumption; and (iii) our definition of excessive alcohol consumption was based on number of drinks per day (four or more drinks per day) which may be an approximation, as we did not have complete data on the specific amount of alcohol consumed by study participants.

In conclusion, available data suggest that there are age interactions in the association of NAFLD (as assessed by FLI or HSI) with the risk of CVD in a general Caucasian population. Further study is required to replicate these findings and understand the mechanisms responsible for the age differences in the associations.

Conflicts of interest

None to declare.

Authorship

SKK, SJLB, HB and RPFD conceived and designed the study. SJLB, HB, and RPFD acquired data. SKK analyzed and interpreted the data. SKK drafted the manuscript. SKK, SJLB, HB and RPFD critically revised the manuscript for important intellectual content. RPFD supervised the study. SKK is the guarantor of this work, and as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

Ethics

This study protocol was evaluated by the ethical committee of our institution. A written consent was obtained from each patient evaluated in this study.

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Table 1 Baseline participant characteristics according to categories of the fatty liver index (FLI)

	< 30 (N=3,298) Mean (SD) or median (IQR) or n (%)	**30 to < 60 (N=1,500) Mean (SD) or median (IQR) or n (%)**	**≥ 60 (N=1,542) Mean (SD) or median (IQR) or n (%)**	***P*-value**
Questionnaire				
Male	1,035 (31.4)	891 (59.4)	1,017 (66.0)	< 0.001
Age at survey (years)	43.9 (11.2)	51.5 (12.9)	53.2 (11.6)	< 0.001
History of diabetes	16 (0.5)	43 (2.9)	118 (7.7)	< 0.001
Smoking				
Current	1,094 (33.1)	456 (30.4)	503 (32.6)	< 0.001
Former	1,034 (31.4)	602 (40.1)	634 (41.1)	-
Never	1,170 (35.5)	442 (29.5)	405 (26.3)	-
Moderate drinkers	2,523 (76.5)	1,108 (73.9)	1,084 (70.3)	< 0.001
History of hypertension	119 (3.6)	168 (11.2)	292 (18.9)	< 0.001
Regular use of anti-hypertensive medication	153 (4.6)	187 (12.5)	296 (19.2)	< 0.001
Physical measurements				
BMI (kg/m^2)	23.3 (2.4)	26.8 (2.4)	30.7 (4.1)	< 0.001
WHR	0.82 (0.07)	0.90 (0.07)	0.96 (0.08)	< 0.001
WC	78.2 (7.8)	91.6 (6.3)	102.9 (9.2)	< 0.001
SBP (mmHg)	120 (17)	132 (19)	140 (19)	< 0.001
DBP (mmHg)	70 (9)	75 (9)	79 (9)	< 0.001
Lipid markers				
Total cholesterol (mmol/l)	5.24 (1.02)	5.87 (1.09)	6.14 (1.08)	< 0.001
HDL-C (mmol/l)	1.51 (0.40)	1.23 (0.32)	1.08 (0.29)	< 0.001
Triglycerides (mmol/l)	0.88 (0.68-1.12)	1.28 (1.00-1.70)	1.89 (1.37-2.69)	< 0.001
Metabolic, inflammatory, liver, and renal function markers				
hsCRP (mg/l)	0.72 (0.34-1.75)	1.49 (0.73-3.13)	2.31 (1.15-4.79)	< 0.001
Fasting plasma glucose (mmol/l)	4.47 (0.57)	4.90 (1.04)	5.35 (1.57)	< 0.001
Creatinine (µmol/l)	78 (71-86)	85 (76-94)	87 (77-96)	< 0.001
Cystatin C (mg/dl)	0.75 (0.18)	0.82 (0.22)	0.84 (0.18)	< 0.001
UAE (mg/24 hours)	7.90 (5.78-12.29)	9.38 (6.41-16.85)	13.21 (7.87-29.45)	< 0.001
Total bilirubin (µmol/l)	7 (5-9)	7 (5-9)	6 (5-9)	< 0.001

BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; ; hsCRP, high sensitivity C-reactive protein; IQR, interquartile range; SBP, systolic blood pressure; UAE, urinary albumin excretion; WC, waist circumference; WHR, waist-to-hip ratio

Table 2 Baseline participant characteristics according to categories of the hepatic steatosis index (HSI)

	< 30 (N=1,460) Mean (SD) or median (IQR) or n (%)	**30 to ≤ 36 (N=2,897) Mean (SD) or median (IQR) or n (%)**	**> 36 (N=1,983) Mean (SD) or median (IQR) or n (%)**	***P*-value**
Questionnaire				
Male	652 (44.7)	1,317 (45.5)	974 (49.1)	0.013
Age at survey (years)	43.2 (11.4)	48.4 (12.6)	50.9 (11.9)	< 0.001
History of diabetes	4 (0.3)	43 (1.5)	130 (6.6)	< 0.001
Smoking				
Current	582 (39.9)	919 (31.7)	552 (27.8)	< 0.001
Former	400 (27.4)	1,076 (37.1)	794 (40.0)	-
Never	478 (32.7)	902 (31.1)	637 (32.1)	-
Moderate drinkers	1,144 (78.4)	2,213 (76.4)	1,358 (68.5)	< 0.001
History of hypertension	39 (2.7)	226 (7.8)	314 (15.8)	< 0.001
Regular use of anti-hypertensive medication	55 (3.8)	244 (8.4)	337 (17.0)	< 0.001
Physical measurements				
BMI (kg/m^2)	21.5 (1.7)	25.1 (2.0)	30.3 (3.9)	< 0.001
WHR	0.82 (0.08)	0.86 (0.09)	0.92 (0.09)	< 0.001
WC	76.1 (8.4)	85.5 (9.6)	98.6 (11.2)	< 0.001
SBP (mmHg)	118 (16)	127 (20)	135 (19)	< 0.001
DBP (mmHg)	69 (9)	73 (10)	77 (9)	< 0.001
Lipid markers				
Total cholesterol (mmol/l)	5.18 (1.01)	5.60 (1.12)	5.93 (1.10)	< 0.001
HDL-C (mmol/l)	1.50 (0.41)	1.37 (0.39)	1.18 (0.34)	< 0.001
Triglycerides (mmol/l)	0.89 (0.69-1.18)	1.08 (0.81-1.52)	1.46 (1.05-2.15)	< 0.001
Metabolic, inflammatory, liver, and renal function markers				
hsCRP (mg/l)	0.60 (0.28-1.54)	1.07 (0.50-2.37)	2.04 (0.99-4.29)	< 0.001
Fasting plasma glucose (mmol/l)	4.42 (0.61)	4.69 (0.81)	5.20 (1.48)	< 0.001
Creatinine (µmol/l)	79 (72-88)	82 (73-91)	84 (74-93)	< 0.001
Cystatine C (mg/dl)	0.75 (0.18)	0.79 (0.20)	0.81 (0.19)	< 0.001
UAE (mg/24 hours)	8.03 (5.87-12.94)	8.77 (6.08-14.62)	10.68 (6.85-21.46)	< 0.001
Total bilirubin (µmol/l)	7 (6-10)	7 (5-9)	6 (5-8)	< 0.001

BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; ; hsCRP, high sensitivity C-reactive protein; IQR, interquartile range; SBP, systolic blood pressure; UAE, urinary albumin excretion; WC, waist circumference; WHR, waist-to-hip ratio

Table 3 Association of fatty liver index (FLI) with cardiovascular disease

FLI categories	Events/ Total	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
< 30	174 / 3,298	ref		ref		ref		ref	
30 to < 60	187 / 1,500	1.12 (0.90 to 1.40)	0.290	0.84 (0.67 to 1.06)	0.145	0.85 (0.67 to 1.07)	0.164	0.82 (0.65 to 1.04)	0.102
≥ 60	270 / 1,542	1.53 (1.25 to 1.88)	< 0.001	0.89 (0.70 to 1.13)	0.354	0.86 (0.68 to 1.10)	0.234	0.85 (0.65 to 1.11)	0.226

CI, confidence interval; HR, hazard ratio

Model 1: Age and sex

Model 2: Model 1 plus smoking status, history of diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein-cholesterol

Model 3: Model 2 plus alcohol consumption, glucose, and $\log_e$ urinary albumin excretion

Model 4: Model 3 plus $\log_e$ total bilirubin, $\log_e$ high sensitivity C-reactive protein, and $\log_e$ homeostasis model assessment-estimated insulin resistance

Table 4 Association of hepatic steatosis index (HSI) with cardiovascular disease

HSI categories	Events/ Total	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
< 30	93 / 1,460	ref		ref		ref		ref	
30 to ≤ 36	279 / 2,897	1.04 (0.82 to 1.31)	0.770	0.83 (0.65 to 1.06)	0.127	0.79 (0.61 to 1.04)	0.090	0.83 (0.64 to 1.08)	0.172
> 36	259 / 1,983	1.29 (1.02 to 1.65)	0.035	0.84 (0.65 to 1.09)	0.180	0.73 (0.51 to 1.05)	0.090	0.79 (0.55 to 1.13)	0.204

CI, confidence interval; HR, hazard ratio

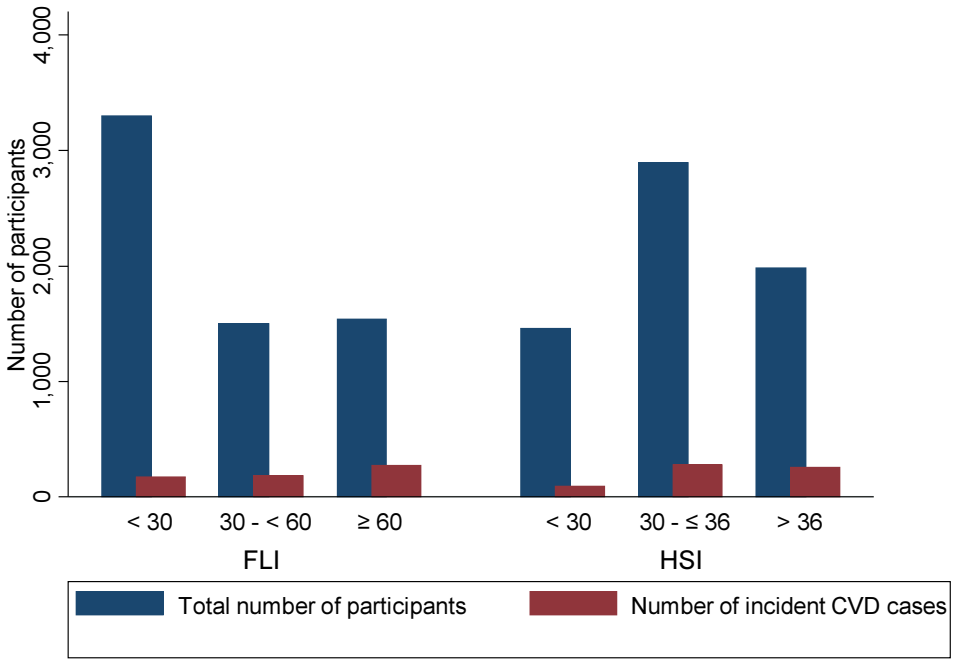
Model 1: Age and sex

Model 2: Model 1 plus smoking status, systolic blood pressure, total cholesterol, and high-density lipoprotein-cholesterol

Model 3: Model 2 plus alcohol consumption, glucose, and $\log_e$ urinary albumin excretion

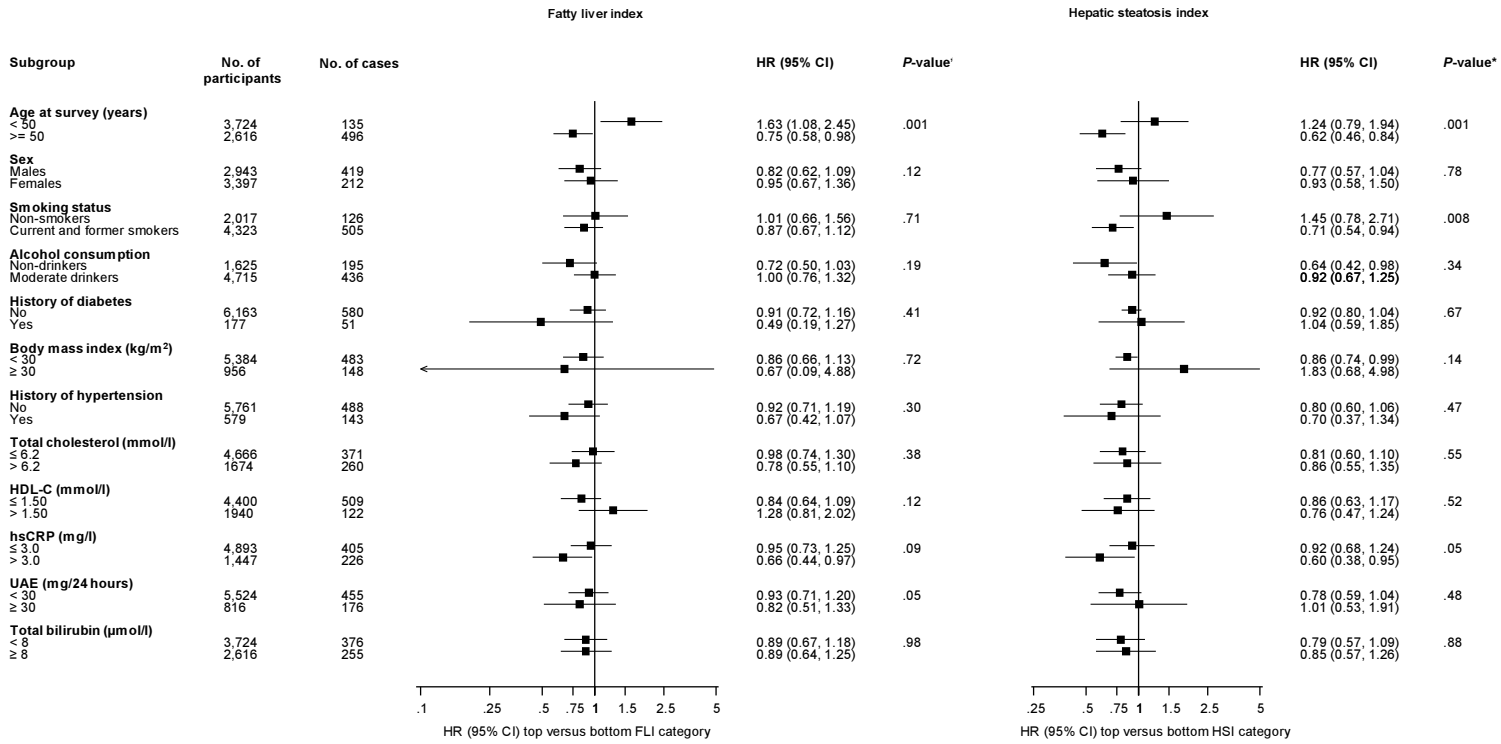
Model 4: Model 3 plus $\log_e$ triglycerides, $\log_e$ total bilirubin, $\log_e$ high sensitivity C-reactive protein, and $\log_e$ homeostasis model assessment-estimated insulin resistance

Figure 1. Proportions on incident cardiovascular disease cases by categories of FLI and HSI



CVD, cardiovascular disease; FLI, fatty liver index; HR, hazard ratio; HSI, hepatic steatosis index

Figure 2 Hazard ratios for FLI, HSI, and cardiovascular disease risk by several participant level characteristics



Hazard ratios were adjusted for age, sex, smoking status, history of diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein cholesterol (HDL-C); CI, confidence interval (bars); hsCRP, high sensitivity C-reactive protein; FLI, fatty liver index; HR, hazard ratio; HSI, hepatic steatosis index; UAE, urinary albumin excretion; *, *P*-value for interaction; cut-offs used for total bilirubin are median values.